#24

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Nikolaus THERES

Serial No.: 09/403,262

Filed: October 15, 1999

For: PLANTS WITH CONTROLLED SIDE-SHOOT FORMATION AND/OR CONTROLLED ABSCISSION ZONE FORMATION

Group Art Unit: 1638

Examiner: Ashwin Mehta

Atty. Dkt. No.: DEBE:016US/SLH

CERTIFICATE OF MAILING
37 C.F.R §1.8

I hereby certify that this correspondence is being deposited with the U.S. Postal Service with sufficient postage as First Class Mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the date below:

May 12, 2003
Date

Steven L. Highlander

DECLARATION OF DR. NIKOLAUS THERES UNDER 37 C.F.R. §1.132

I, Nikolaus Theres, do declare that:

1. I am a citizen of Germany and reside at Schiffgesweg 30, 50259 Pulheim, Germany. I am a named inventor of the above-captioned application.

2. I received my Ph.D. in natural sciences from the University of Cologne, Germany, in 1986. From 1986 to 1988, I spent two years as a postdoctoral fellow in the Institute of Genetics, University of Cologne, Germany. From 1989 to 1998, I was the head of an independent research group at the Institute of Genetics, University of Cologne, Germany.

Since 1998, I have been working as a group leader at the Max-Planck-Institute for Plant Breeding Research, Cologne, Germany.

3. My major research interest is molecular plant genetics, combining state-of-the-art molecular biology methods with classical genetics techniques. Since 1986, I have been working in the field of plant transposable elements with significant contributions to the development of transposon tagging strategies. Since 1990, my work is focused on the developmental biology of seed plants with a special emphasis on the analysis of shoot architecture. In the above-mentioned fields, I have published more than 20 research papers and book articles and I have presented over 15 abstracts at scientific meetings. A copy of my *curriculum vitae* and a list of publications is attached.

4. I understand that the examiner for the above-captioned application has questioned whether one of skill in the art could make various modifications to the polypeptide encoded by SEQ ID NO:2, known as the *Lateral suppressor* gene product, while retaining the function of that molecule, *i.e.*, increased shoot formation, petal formation, and abscission zone formation. A number of experiments have been performed which I believe demonstrate that this is the case.

4. As experiment has been performed where an HA tag (11 amino acids in length) has been inserted in the NH terminus of the Ls gene product. More specifically, a 5.6 kb XhoI-SnaBI DNA fragment comprising the base pairs 1 to 5570 of cosmid G (Rossberg *et al.*, 2001, *Plant Cell* 13:979-988; EMBL accession no. AJ303345) was subcloned into the plasmid vector pBluescript SK+. The XhoI site used was located in the vector part (pCLD04541; accession no. AF184978) of cosmid G. This DNA fragment contained the

complete open reading frame of the tomato *Lateral suppressor* gene (bp 2903-4189, EMBL accession no. AJ303345, reverse strand), as well as about 1.4 kb of 5′-sequence and about 2.9 kb of 3′-sequence. The *Lateral suppressor* gene was modified by the insertion of an oligonucleotide, encoding an HA tag (Fields *et al.*, 1988, *Mol. Cell. Biol.* 8:2159-2165), into a BamHI cleavage site at the beginning of the *Lateral suppressor* open reading frame (bp 4178; EMBL accession no. AJ303345). This modification resulted in the insertion of a total of 33 basepairs, encoding the amino acids SYPYDVPDYAR. This construct was transferred into the binary vector pGPTV-Kan (Becker *et al.*, 1992, *Plant Mol. Biol.* 20:1195-1197) and introduced via *Agrobacterium*-mediated transformation into the tomato lateral suppressor (ls^1/ls^1) mutant.

5. Fourteen transgenic tomato lines containing at least one copy of the construct were analyzed for side-shoot and petal development. Of the 14 transgenic lines, four developed side-shoots in every leaf axil of the primary shoot, and a complete whorl of petals on the flowers. Five additional lines developed side-shoots in more than 60% of the leaf axils of the primary shoot, and a nearly complete whorl of petals on the flowers. The remaining 5 lines showed a low degree of side-shoot and petal development. This result demonstrates that the modified gene has retained the biological activity of the *Lateral suppressor* gene.

6. Another experiment looked at the ability of a homologous *Lateral suppressor* gene product from *Arabidopsis* to complement a defect in a tomato *Lateral suppressor* gene product. Sequence analysis revealed that the *Arabidopsis Lateral suppressor* gene (*LAS*) encodes a protein with 50.5% identity to the orthologous gene from tomato (Ls). To test

for functional complementation between these two distantly related plant species, a 6.3 kb NheI-BanI DNA fragment (bac T5A14, position 3404 to 9740, reverse strand; Genbank accession no. AC005223; Rossberg *et al.*, 2001, *Plant Cell* 13:979-988), was cloned into the binary vector pGPTV-Kan (Becker *et al.*, 1992, *Plant Mol. Biol.* 20:1195-1197) and introduced via *Agrobacterium*-mediated transformation into the tomato lateral suppressor (ls^1/ls^1) mutant. The introduced DNA fragment contained the complete open reading frame of the *LAS* gene, as well as about 2.9 kb 5'-sequence and 2.1 kb 3'-sequence.

7. Four independent transgenic lines harbouring at least one complete copy of the *LAS* gene were established. Two transgenic lines developed side-shoots in almost every leaf axil and a whorl of petals as well as abscission zones on all flowers. The two additional lines developed side-shoots in only a fraction of their leaf axils, and also showed an incomplete restoration of the wild-type flower phenotype. Inheritance of the complementation phenotype was analyzed in one transgenic line harbouring a single copy T-DNA insertion. Among 19 plants of the self-pollinated progeny, 14 plants showed complementation, and 5 plants had the *Lateral suppressor* phenotype. By Southern analysis, the T-DNA was detected only in those plants showing complementation. This result is consistent with the assumption that a single-copy T-DNA insertion, segregating in a Mendelian fashion, rescues the *Lateral suppressor* phenotype.

8. I also understand that the examiner has questioned enablement of the claimed sequences based upon the absence of a phenotype for the corresponding transgenic plant. Again,

however, data bearing on this issue shows that overexpression of the Ls gene gave the expected phenotype.

9. An experiment has been performed using the powerful cauliflower mosaic virus enhancer element to drive expression of the *Lateral suppressor* gene product using the endogenous *Lateral suppressor* promoter. Two copies of the EcoRV-HincII fragment of the cauliflower mosaic virus promoter (bp 7344-7018 of the cauliflower mosaic virus genome; accession no. V00141) containing the strong cauliflower mosaic virus enhancer were inserted in front of a 5.6 kb SnaBI-XhoI DNA fragment of cosmid G containing the complete tomato *Lateral suppressor* open reading frame as well as about 1.4 kb of 5′-sequence and about 2.9 kb of 3′-sequence (see above). This construct was transferred into the binary vector pZP212 (accession no. U10462) and introduced by *Agrobacterium*-mediated transformation into the tomato *Lateral suppressor* mutant (ls^1/ls^1). The transgenic plant obtained from this experiment developed side-shoots in some of their leaf axils and an incomplete whorl of petals on its flowers, indicating a partial complementation. Furthermore, many additional shoots developed from the upper surface of the leaves and the leaf petioles. The result of this experiment suggests that overexpression of the *Lateral suppressor* gene leads to the formation of ectopic shoots during leaf development.

11. I hereby declare that all statements made herein of my knowledge are true, and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the referenced patent application or any patent issued thereon.

04.02.2003
Date

N. Theres
Nikolaus Theres, Ph.D.

Appendix: List of publications

Original papers

Theres N, Scheele T, Starlinger P (1987) Cloning of the *Bz2* locus of *Zea mays* using the transposable element *Ds* as a gene tag. Mol. Gen. Genet. 209:193-197.

Schmitz G, **Theres K** (1992) Structural and functional analysis of the *Bz2* locus of *Zea mays*: characterization of overlapping transcripts. Mol. Gen. Genet. 233:269-277.

Brandstädter J, Roßbach C, **Theres K** (1994) The pattern of Histone H4 expression in the tomato shoot apex changes during development. Planta 192:69-74.

Knapp S, Larondelle Y, Roßberg M, Furtek D, **Theres K** (1994) Transgenic tomato lines containing *Ds* elements at defined genomic positions as tools for targeted transposon tagging. Mol. Gen. Genet. 243:666-673.

Schmitz G, **Theres K** (1994) A self-stabilizing *Ac* derivative and its potential for transposon tagging. Plant J. 6:781-786.

Meissner R, **Theres K** (1995) Isolation and characterization of the tomato homeobox gene THOM1. Planta 195:541-547.

Schumacher K, Ganal M, **Theres K** (1995) Genetic and physical mapping of the *lateral suppressor (ls)* locus in tomato. Mol. Gen. Genet. 246:761-766.

Brandstädter J, Roßbach C, **Theres K** (1996) Expression of genes for a defensin and a proteinase inhibitor in specific areas of the shoot apex and the developing flower in tomato. Mol. Gen. Genet. 252:146-154.

Schumacher K, Schmitt T, Rossberg M, Schmitz G, **Theres K** (1999) The *Lateral suppressor* gene of tomato encodes a new member of the VHIID protein family. Proc. Natl. Acad. Sci. USA 96:290-295.

Suzuki Y, Uemura S, Saito Y, Murofushi N, Schmitz G, **Theres K**, Yamaguchi I (2001) A novel transposon tagging element for obtaining gain-of-function mutants based on a self-stabilizing *Ac* derivative. Plant Mol. Biol. 45:123-131.

Rossberg M*, **Theres K***, Acarkan A, Herrero R, Schmitt T, Schumacher K, Schmitz G, Schmidt R (2001) Comparative sequence analysis reveals extensive microcolinearity in the *Lateral suppressor* regions of the tomato, Arabidopsis, and Capsella genomes. Plant Cell 13:979-988.
*These authors contributed equally to this work.

Burbidge A, Lindhout P, Grieve TM, Schumacher K, **Theres K**, van Heusden AW, Bonnema AB, Woodman KJ, Taylor IB (2001) Re-orientation and integration of the classical and interspecific linkage maps of the long arm of tomato chromosome 7. Theor. Appl. Genet. 103:443-454.

Schmitz G, Tillmann E, Carriero F, Fiore C, Cellini F, **Theres K** (2002) The tomato *Blind* gene encodes a MYB transcription factor that controls the formation of lateral meristems. Proc. Natl. Acad. Sci. USA 99:1064-1069.

Mishra SK, Tripp J, Winkelhaus S, Tschiersch B, **Theres K**, Nover L, Scharf KD (2002) In the complex family of heat stress transcription factors, HsfA1 has a unique role as master regulator of thermotolerance in tomato. Genes & Development 16:1555-1567.

Greb T, Schmitz G, **Theres K** (2002) Isolation and characterization of the *Spindly* homologue from tomato. J. Exp. Botany 53:1829-1830.

Gidoni D, Fuss E, Burbidge A, Speckmann G-J, James S, Nijkamp D, Mett A, Feiler J, Smoker M, de Vroomen MJ, Leader D, Liharska T, Groenendijk J, Coppoolse E, Smit JJM, Levin I, de Both M, Schuch W, Jones JDG, Taylor IB, **Theres K**, van Haaren MJJ (2002) Multi-functional T-DNA/Ds tomato lines designed for gene cloning and molecular and physical dissection of the tomato genome. Plant Mol. Biol. 51:83-98.

Greb T, Clarenz O, Schäfer E, Müller D, Herrero R, Schmitz G, **Theres K** (2003) Molecular analysis of the *LATERAL SUPPRESSOR* gene in *Arabidopsis* reveals a conserved control mechanism for axillary meristem formation. submitted

Review

Schmitz G, **Theres K** (1999) Genetic control of branching in *Arabidopsis* and tomato. Curr. Opin. Plant Biol. 2:51-55.

Contributions to Books

Starlinger P, Courage-Tebbe U, Döring HP, Frommer WB, **Theres K**, Tillmann E, Weck E, Werr W (1984) Isolation of transposable elements in maize. In: Arber W, Illmensee K, Peacock WJ, Starlinger P (Ed.) Genetic manipulation: impact on man and society. ICSU Press, Letchworth, Herfordshire, pp. 67-74.

Theres N, Schmitz G, Scheele T, Starlinger P (1989) The Bz2 locus in maize: Cloning and transcription studies. In: Styles DE, Gavazzi GA, Racchi ML (Ed.) The Genetics of Flavonoids. Edizioni Unicopli, Milano, pp. 97-104.

Theres K, Brandstädter J, Krebs B, Meissner R, Roßberg M, Schmitz G, Schumacher K, Tillmann E (1994) Gene expression in the tomato shoot apex. In: Molecular Biology of the Cell, (Ed.: W. Doerfler), Druckerei Hansen, Köln, pp. 459-467.

Theres K, Hankammer H, Kühn S, Schmitt T, Schmitz G, Schumacher K, Tillmann E (1996) Genetic control of shoot development in tomato. In: Molecular Biology, (Ed.: W. Doerfler), Druckerei Hansen, Köln, pp. 441-446.

Madhuri G, Schmitz G, **Theres K**, Reddy AR (1997) Expression of maize *Bronze2* gene in *E. coli* and detection of *Bz2* specific mRNA in rice (*Oryza sativa* L.). In: Chopra VL, Sharma RP, Swaminathan MS (Hrsg.) Agricultural biotechnology. Science Publishers Inc., Enfield, New Hampshire, pp. 226-232.

Theres K, Hankammer H, Herrero R, Schäfer E, Schmitt T, Schmitz G, Schumacher K, Tillmann E (1998) Genetic control of side-shoot development in higher plants. In: Molecular Biology, (Ed.: W. Doerfler), Druckerei Hansen, Bergisch-Gladbach, pp. 429-435.

Theres K (2002) Gene isolation in tomato. Sonderforschungsbereichs 274 der Deutschen Forschungsgemeinschaft - Abschlußbericht, in press

Selected Abstracts and Presentations (1995-2001)

Theres K (1995) Characterization of genes controlling shoot development in tomato. Frontier Research Forum "Advances in Plant Biohomeostasis Research", Wako-City, Japan, Oral presentation.

Schumacher K, Rossberg M, Schmitt T, Schmitz G, **Theres K** (1996) Positional cloning of candidates for the *Lateral suppressor* gene from tomato. Plant genome IV, The international conference on the status of plant genome research, San Diego, USA.

Schumacher K, Rossberg M, Schmitt T, Schmitz G, **Theres K** (1996) Map-based cloning of the Lateral suppressor gene from tomato. Gatersleben Research Conference, Schloß Meisdorf, Book of abstracts, Oral presentation.

Theres K (1996) Side-shoot development in tomato. Frontier Research Forum "Progress in Tomato Homeostasis Research", Wako-City, Japan, Oral presentation.

Schmitt T, Schmitz G, Schumacher K, Roßberg M, **Theres K** (1997) Die Rolle des Lateral suppressor-Gens in der Sproßentwicklung der Tomate. Tagung "Molekularbiologie der Pflanzen", Wernigerode, Oral presentation.

Schumacher K, Rossberg M, Schmitt T, Schmitz G, **Theres K** (1997) Shoot development in tomato. 6th Symposium of The Otto Warburg Center for Agricultural Biotechnology "Developmental Pathways in Plants: Biotechnological Implications", Rehovot, Israel, Oral presentation.

Schmitt TT, Schumacher K, Roßberg M, Schmitz G, **Theres K** (1997) Molecular and genetic analysis of the Lateral suppressor gene controlling shoot branching and petal development in tomato. 5th International Congress of Plant Molecular Biology, Singapore.

Suzuki Y, Uemura S, Murofushi N, Schmitz G, **Theres K** (1998) A system for preparation of Arabidopsis gain-of-function mutants using Ac/Ds transposon. Annual Meeting of the Japanese Society of Plant Physiologists, Tokyo, Japan. Plant & Cell Physiology 39: 115.

Theres K (1999) Genetic control of shoot branching in tomato. Frontier Research Forum "Progress in Tomato Homeostasis Research II", Wako-City, Japan, Oral presentation.